104-Plat
Ultrastructure of Dynactin Complex: A Mediator of Cytoplasmic Dynein
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The ~1.0 megadalton dynactin complex interacts with cytoplasmic dynein to increase its processivity during minus-end-directed transport of cargo along microtubules (MT). The detailed molecular understanding of how dynactin regulates dynein motility is still elusive due to lack of structural information of the complex. Here we present structure of vertebrate dynactin at 20Å resolution, achieved by negative stain electron microscopy (EM). The reconstruction reveals the overall architecture of dynactin, allowing for delineation of the major known subcomplexes of the molecule. We can clearly discern the individual Arp subunits arranged in actin like helical fashion in the filament, along with the capping density at the barbed-end, and the pointed-end complex. We can also identify the shoulder domain above the filament observing extensive interactions with the Arp subunits. Due to the flexibility of the extended p150Glued coiled-coil arm, whose base interacts with dynein and whose globular tip binds the MT surface, this region was not resolved in the 3D reconstruction. However, focused 2D analysis of the p150Glued arm revealed its attachment point at the shoulder domain, as well as structural details of the globular CAP-Gly domain. This structural study of the dynactin complex establishes a strong foundation for understanding how its architecture is adapted for concerted interaction with dynein, cargo, and MTs during transport processes.

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A Mechanical Switch from Diffusion to Directional Motion Activates ATPase in Dynein Motor
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Dynein is a motor protein that moves along microtubule tracks via the energy from ATP hydrolysis. Unlike other processive cytoskeletal motors, the dynein step size is highly variable with a significant level of diffusion. To investigate the molecular basis of the stochastic nature of dynein stepping, we here characterized the structure, physical properties, and effects of site-directed mutations of the dynein-microtubule interface.
We found that mutation of either the R403 or E416 residue of α-tubulin to alanine changed the directional movement of the microtubules on a dynein-coated surface to undirected thermal diffusion, resulting in a loss of dynein ATPase activity. Biochemical and cryo-electron microscopy analyses of the microtubule binding domain (MTBD)-microtubule complex revealed that these tubulin residues switch dynein from diffusional to stationary binding by forming salt bridges with the residue in H1 and H6 of the MTBD. The formation of two salt bridges then triggers a registry change in the stalk coiled coil required for ATPase activation, and thus leads to directional movement. In this mechanism, the previously undescribed interaction between α-R403 and E3390 in H1 of the MTBD plays a key role, and is likely to explain the fact that the equivalent tubulin mutation in mammals (R402) can cause lissencephaly (Keays et al., *Cell* **128,** 45-).
Compared to kinesin-microtubule interactions, where the weak-to-strong state transition is mediated by several contact sites involving a few tubulin residues (Uchimura et al., *EMBO. J.*, **29,** 1167-), for dynein, the mechanical switch from diffusional to stationary binding is controlled by only two salt bridges. Because of this pinpoint regulation, the stepping motion of dynein might be only loosely coupled with the reaction of ATP hydrolysis, resulting in the variable step size.

106-Plat
Cytoplasmic Dynein Ring Tilting Detected by Combined polTIRF and Sub-Pixel Particle Tracking of Semiconductor Quantum Rods
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The mechanism of cytoplasmic dynein, a microtubule-based motor responsible for the majority of minus-end-directed transport in eukaryotes, remains poorly understood compared to myosin and kinesin. While recent crystal and EM structures have given insight into the conformational changes that occur in the AAA+ ring of dynein during ATP hydrolysis, dynamic structural information is lacking. In order to better understand the dynein translocation mechanism we combine polarized total internal reflection fluorescence (polTIRF) microscopy and high precision localization to simultaneously track position and orientation of single dynein molecules in real time. CdSe/CdS rod-in-rod nanoparticles with polarized emission are coated with mercaptoundecanoic acid (MUA) and functionalized with NeutrAvidin. The polarized nanorods are bifunctionally conjugated via biotin-avidin linkages to biotinylation sites inserted in two positions in dynein's AAA+ ring. Both homo- and hetero-dimeric dynein constructs are analyzed. Fluorescence emission of dynein-conjugated rods is split into four channels based on polarization and imaged with an EMCCD camera. The relative intensities of the four channels are used to determine the three-dimensional orientation of the rod, and therefore the dynein AAA+ ring, during stepping. Using this combined polTIRF/tracking method we detect tilting of the ring domain during stepping. Ring rotations are ATP-dependent, highly irregular and are mainly in the plane of the microtubule when correlated with translocation events. Supported by NIH grant P01GM087253.

107-Plat
Bidirectional Helical Motility of Cytoplasmic Dynein around Microtubules
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Cytoplasmic dynein is a molecular motor responsible for minus-end-directed cargo transport along microtubules (MTs). Dynein motility has previously been studied on surface-immobilized MTs in vitro, which constrains the motors to move in two dimensions. In this study, we explored dynein motility in three dimensions using an MT bridge assay. We found that dynein moves in a helical trajectory around the MT, demonstrating that it generates torque during cargo transport. Unlike other cytoskeletal motors that produce torque in a specific direction, dynein generates torque in either direction, resulting in bidirectional helical motility. Dynein has a net preference to move along a right-handed helical path, suggesting that the heads tend to bind to the closest tubulin binding site in the forward direction when taking sideways steps. This bidirectional helical motility may allow dynein to avoid roadblocks in dense cytoplasmic environments during cargo transport.

Symposium: Systems Biology Approaches in Neuroscience

108-Symp
Mapping Behavior to Neural Anatomy Using Machine Vision and Thermogenetics
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To understand the relationship between neural anatomy and behavior, the ultimate output of the nervous system, we performed a high-throughput, thermogenetic screen of 2,200 transgenic Drosophila lines from the Janelia GAL4 collection. Each GAL4 line drives expression in a different, sparse subset of neurons in the fly nervous system. Using genetic techniques, we selectively activated these sparse subsets of neurons, and measured the behavioral effects. We developed a high-throughput, automated system for measuring the flies' locomotion and social behavior with breadth and depth. We recorded 20,000 videos of groups of flies freely behaving in an open-field walking arena, totaling ~400 TB of raw data. From the video, we tracked the flies' body and wing positions using our tracking software, Ctrax. We used our machine learning-based behavior classification system, JAABA, to create 15 behavior classifiers (e.g. walking, chasing) that input trajectories from Ctrax and output predictions for each frame of each fly's behaviors (totaling ~175 billion annotations of behavior). For each line of flies, we computed ~200 behavior statistics, such as the fraction of time spent chasing, or average speed while walking, summarizing the behavioral effects of activating the targeted neurons in a concise, interpretable manner. Concurrent with our screen, the